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January 13, 1947.

Dear Prof. Boivin-

Prof. Tatum and I have read your provocative letter with deepest interest. We hope that you shall be able to resume your researches with despatch, and that you will be kind enough to let us know how things are going.

As our publication in Nature suggests, we are under the conviction that the new types which we have found in bacterial mutants are the result of a process of gene recombination, rather than a transfer of genetic material through the medium, as you demonstrated for the antigenic characters of coli C1 and C2. We designate our strains by various symbols according to ~~whether~~ whether they are (+) or are not (-) capable of synthesizing various growth factors, such as: biotin (B); methionine (M); threonine (T) leucine (L); thiamin (B_1); phenylalanine (\emptyset); proline (P); cystine (C), etc. There are also the characters of lactose fermentation (lac) and of resistance to the bacteriophage T1 (T_1^r). In this letter we hope to elaborate some of the reasons for the convictions that gene recombination occurs:

If young (6-hour) cultures of B-M-T+L+B₁+ and of B+M+T-L-B₁-

~~xxxxxx~~ (grown separately in nutrient broth) are washed with water, mixed, and about 10^8 cells are plated into minimal agar, (containing no growth factors) 100 colonies of prototrophs (B+M+T+L+B₁+) appear the next day. These prototrophs are stable and we have good reason to believe that they are indeed a new cell type. Therefore, within a few hours after the mutant cells are mixed, prototrophic cells must occur, which then form colonies in the minimal agar. We have looked for transforming factors in minimal medium in which washed cells have been suspended for 3-4 hours, by simply removing the cells substantially by centrifugation. Nothing was found in the medium; only by mixing the cells with the other mutant type could prototrophs be obtained. Similar negative results have been obtained with the use of extracts prepared in the manner you described. (These were simply the crude autolysate, freed of the mutant cells by centrifugation, and added into agar with the other mutant type.) Other indirect evidence that transformation plays no role under our experimental conditions is the following:

1. Comparisons of the relative number of prototrophs and types such as B+M+T-L-B₁+. The former are much more frequent (10^4 .) If these are to be accounted for by transformation, it would mean that the three factors T-, L-, B₁- are more often transformed all at once than simply two factors such as T- and B₁-.

2. The occurrence of types such as B-M+T+L-B₁+. If this came from B-M-T+L+B₁+, it would mean that M- became M+, and that L+ became L-. Many comparable types have been obtained, leading to the conclusion that if transformation occurs, it can change a factor from - to +, or from + to -.

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3. A limited number of experiments where a mixture of three types was used, such as: B+T-L- & B-T+L- & B-T-L+. On the transformation theory, one would expect to find B+T+L+ in such a mixture. This has not been found. On the gene-recombination theory, cells would react two at a time: leading to the types: B+T+L-, B+T-L+ and B-T+L+, but not to B+T+L+.

Particularly on the basis of the above experiments, we have been led to the belief that gene recombination does occur in our material. We are pursuing an attempt to discover conditions for transformation, but have so far been unsuccessful.

For the understanding of the genetic basis of bacterial 'mutations dirigées' it would be extremely interesting to observe the results of the following types of experiments using biochemical mutants of appropriate strains such as C1 and C2. Let X be the biochemical factor.

Transform(C2R-X-) to C1S.. with an extract of C1S-X+. This would establish the scope of transformation, by examining the C1S that were obtained as to X- or X+. It would also provide information of the transformation of independent factors.

Transform(C2R-X+) to C1S.. with an extract of C1S-X-. This would provide critical evidence as to the possibility of mutant genes directed the mutation of other genes to the same state.

If these genetic problems are of interest to you, we should be happy to be of any service that we can in their elucidation, in any manner that you may care to suggest.

With best regards from Professor Tatum,

Sincerely yours,

Joshua Lederberg.